Cell reprogramming

More than one way to induce a neuron

Seventy-six pairs of transcription factors can induce connective-tissue cells from mice to adopt a neuron-like identity *in vitro*. This discovery provides insights into both neuronal development and cell reprogramming. See Article p. XXX

Lynette Lim & Oscar Marín

The brain contains hundreds of neuronal subtypes, each defined by a specific combination of features, including its position and shape, the neurotransmitter molecules it produces and its electrophysiological properties[1]. Engineering this enormous diversity in the laboratory is an ultimate goal of regenerative medicine. In a paper in *Nature*, Tsunemoto *et al.*[2] describe the results of a large-scale effort to identify factors that can endow non-neural cells cultured *in vitro* with neuronal properties.

Understanding the mechanisms that underlie the generation of neuronal diversity has been a central goal of neurobiology for more than a hundred years, since Santiago Ramón y Cajal postulated that the nervous system is made up of discrete individual cells[3]. Work over the past three decades has identified gene regulatory networks that control neuronal identity as it unfolds progressively in the embryonic brain[4]. These studies have also revealed that neuronal identity is intimately linked to the environment in which neurons develop, primarily because some of the most important features of neurons, such as their connections, depend on their interaction with other neuronal cells.

In the past decade, however, it has become clear that many neuronal attributes can be generated outside the normal context of brain development. For example, in 2010, it emerged[5] that a cocktail of three transcription factors can be applied to fibroblasts (the most common cells of connective tissue) cultured *in vitro* to convert them into cells that resemble brain-derived neurons, at least in terms of their shape and electrophysiological properties. This procedure, called direct lineage reprogramming, is based on the premise that the chosen transcription factors regulate the expression of genes that are characteristic of neuronal cell types. But what has been unclear is whether the capacity to reprogram cells into neurons is limited to only a handful of transcription factors.

Previous work by the group that performed the current study showed that a pair of transcription factors from the basic helix-loop-helix (bHLH) and Pit-Oct-Unc (POU) families can induce neuronal marker expression through direct reprogramming[6]. Tsunemoto *et al.* were inspired by this finding in their current work. The authors screened 598 bHLH and POU transcription-factor pairs — chosen based on their expression in neuronal lineages — to see which could transform mouse embryonic fibroblasts into neurons *in vitro* (Fig. 1). 76 of the pairs produced cells that expressed multiple markers of mature neurons and had neuronal morphologies. Thus, neuronal features can be induced in non-neuronal cells by an astonishing range of transcription-factor combinations.

How similar are the neurons induced by the different combinations of transcription factors? Analysis of gene expression in individual cells using single-cell RNA sequencing revealed that a given pair of factors generates relatively homogenous

populations of neurons, which share a similar molecular profile. This is surprising, because previous experiments have highlighted the heterogeneity of cell populations undergoing reprograming in culture[7].

By contrast, Tsunemoto *et al.* found that different transcription-factor combinations induced distinct neuron-like populations with characteristic markers and electrophysiological features. However, they also found that certain features — such as the expression of particular ion channels or neurotransmitter receptors — could be induced by multiple combinations of transcription factors. These findings support the notion that there is not a single 'gene code' for making a particular feature of neuronal identity, but rather that the molecular machinery underlying neuronal development is built with a remarkable degree of redundancy. In other words, identity-defining transcription factors are likely used in different combinations in distinct neuron types.

One key question is whether the induced neurons faithfully mimic neuronal cell types found *in vivo*, or whether (and to what extent) they represent somewhat artificial cell types. To address this issue, the authors compared gene-expression patterns in induced cell populations with endogenous mature neuronal subtypes from mice. This analysis indicated that the induced neurons do not match endogenous cell types of the adult mouse brain. However, it might be that the induced neurons did not reach the same stage of development than endogenous cells. Matching cell types across different developmental stages remains a complex problem in neurobiology, as illustrated recently for inhibitory neurons in the cerebral cortex[8,9]. These single-cell transcriptomic studies highlighted the difficulty of recognising identity-defining transcription factors at very early stages of neuronal development. It is possible, then, that the neurons induced

in vitro by Tsunemoto *et al.* correspond to specific populations of endogenous neurons in a relatively immature state. These neurons might develop into fully differentiated neurons only if placed in the appropriate environment. Alternatively, the expression of a pair of transcription factors might be sufficient to elicit some neuronal features in fibroblasts, but not to unleash the complete programme of differentiation that takes place in the embryo.

Regardless, Tsunemoto and colleagues' study provides further evidence that some features of neuronal identity can be reproduced outside of the developing brain, and so illustrates the power of reprogramming to interrogate the function of neuron-specific genes. The authors have made their findings available in a database (http://biogps.org/dataset/BDS_00016/) that will enable other researchers to use the transcription-factor codes to induce specific neuronal features. This will doubtless prove useful for studying the selective vulnerability of specific neuronal subtypes to disease.

Finally, the authors provide preliminary evidence that their transcription-factor combinations can also be used to generate neurons from human fibroblast-like cells. Following further validation, the codes might therefore help us to decipher the origins of neuronal diversity in humans.

Lynette Lim *and* **Oscar Marín** *are in the Centre for Developmental Neurobiology, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London SE1 1UL, UK and in the MRC Centre for Neurodevelopmental Disorders, King's College London.*

e-mail: oscar.marin@kcl.ac.uk

1. Zeng, H. & Sanes, J. R. Neuronal cell-type classification: challenges, opportunities and the path forward. *Nat. Rev. Neurosci.* **18**, 530-546 (2017).
2. Tsunemoto, R. *et al.* Diverse reprogramming codes for neuronal identity. *Nature* **XX**, XX-XX (2018).
3. Ramón y Cajal, S. *Histologie du Systéme Nerveus de l'Homme et des Vestrébrés*. (Maloine, A., 1909).
4. Lodato, S. & Arlotta, P. Generating neuronal diversity in the mammalian cerebral cortex. *Annu. Rev. Cell. Dev. Biol.* **31**, 699-720 (2015).
5. Vierbuchen, T. *et al.* Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* **463**, 1035-1041 (2010).
6. Blanchard, J. W. *et al.* Selective conversion of fibroblasts into peripheral sensory neurons. *Nat. Neurosci.* **18**, 25-35 (2015).
7. Treutlein, B., *et al.* Dissecting direct reprogramming from fibroblast to neuron using single-cell RNA-seq. *Nature* **534**, 391-395 (2016).
8. Mi, D. *et al.* Early emergence of cortical interneuron diversity in the mouse embryo. *Science*, doi: 10.1126/science.aar6821 (2018).
9. Mayer, C. *et al.* Developmental diversification of cortical inhibitory interneurons. *Nature*, doi:10.1038/nature25999 (2018).

Figure 1 | Inducing neurons through direct reprogramming. Tsunemoto *et al.*[2] grew embryonic fibroblasts (connective-tissue cells isolated from mouse embryos) *in vitro*. They treated the cells with pairs of transcription factors, one from each of the

basic helix-loop-helix (bHLH) and Pit-Oct-Unc (POU) families, which are expressed in neurons *in vivo*. In total, more than 12% of the transcription-factor combinations tested could reprogram the fibroblasts into cells that had neuronal properties. Different pairs produced neurons-like cells that had different shapes, gene-expression profiles and electrophysiological properties (indicated by different colours).